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FY 1995 ANNUAL REPORT

Veterinary Resources Program

National Center for Research Resources

National Institutes of Health

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VETERINARY RESOURCES PROGRAM

NATIONAL CENTER FOR RESEARCH RESOURCES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FOR FY 1995

MELVIN B. DENNIS, JR., D.V.M.
ACTING DIRECTOR

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VETERINARY RESOURCES PROGRAM PROJECTS (Z01 RR)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00001-25 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Model Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to develop models of human diseases in small animals, primarily mice and rats. The focus has been on those diseases which have a complex genetic etiology and which also include a significant environmental component. In the rat, the emphasis has been on models for the study of rheumatoid arthritis, insulin independent diabetes mellitus, behavioral disorders as well as chemical addictions. In the mouse, models for the study of human immune disorders have been developed by making different combinations of single mutations which affect different components of the murine immune system.

A secondary but critical aspect to the above studies has been the evaluation of those components which might affect reproductive efficiency. Results thus far indicate that reproductive efficiency of mice and hamsters are sensitive to seasonal differences whereas species such as rats, guinea pigs and rabbits appear be immune to these effects.

OBJECTIVES:

To develop and maintain models of human disease in small animals for the use by the biomedical research community.

METHODOLOGY

Selective breeding is the primary means of developing these models. Only those individuals who are known to genetically carry the trait in question are allowed to reproduce.

MAJOR FINDINGS:

The nature of this project only allows incremental results. The project which has been underway for many years has yielded many useful models which have been utilized in a broad spectrum of biomedical research. For example, models have been developed which are used for gene therapy studies (RHA/N-j), Experimental Autoimmune Uveoretinitis in the rat (LEW/SsN-rnu), rat models for the study of Insulin Independent Diabetes Mellitus (LA/N-cp, SHR/N-cp and now more recently, IRL/N-cp rat congenics), mouse models appropriate for the study of autoimmune diseases to include a model (N;NIH-bnx) which is almost completely immunoincompetent as well as others.

SIGNIFICANCE:

The result of this project has been the creation of a internationally recognized resource for genetically defined animal models of human disease. The product of this effort are used world-wide for a broad spectrum of research activities.

PROPOSED COURSE:

Continuation.

PUBLICATIONS:

Glowa JR, Hansen CT. Differences in response to an acoustic startle response among 46 rat strains, Behavior Genetics 1994;6:79-82.

Caspi RR, Chan C, Fujino S, Grover S, Silver PB, Hansen CT, Wilder RL, Recruitment of naive T cells plays a pivotal role in experimental uveoretinitis (EAU), Regional Immunology 1994;6:24-6.

Michaelis OE, Velasquez MT, Abraham AA, Servetnick DA, Scholfield DJ, Hansen CT. Development and characteristics of a new strain of obese hyperinsulinemic and hyperlipidemic dahl dahl salt sensitive/NIH-corpulent rat, American Journal of Hypertension 1995;8:467-73.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-00077-11 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an Embryo Cryopreservation Program in Laboratory Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Patricia M. Schmidt, M.S.	Physiologist	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
Xi Lin, D.D.S.	Biologist	GRS, SSB, VRP, NCRR
William F. Rall, Ph.D.	NRC Senior Research Associate	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH Animal Genetic Resource (NIHAGR) maintains several animal species and many invaluable genotypes within a species, which are used as models in biomedical research. To protect this germ plasm, an embryo bank was established as an economical means to indefinitely preserve these stocks and strains of rodents and rabbits. Cryopreservation of embryos has other benefits for genotype management such as safeguarding against loss of germ plasm from disease, reproductive failure, natural catastrophe or genetic contamination. The cryobank minimizes changes due to genetic drift by providing a resource from which to periodically rederive defined genotypes for restandardization of foundation breeding colonies. As a recognized repository for laboratory animal species, the NIHAGR routinely provides breeding nuclei upon request throughout the biomedical research community. Rather than shipping live animals, cryopreserved embryos represent a simpler, more humane and easier method to transport requested germ plasm worldwide. Another important impact of embryo handling, cryopreservation, and transfer procedures is to eliminate the vertical transmission of some murine diseases.

OBJECTIVES:

The objective is to study the development and cryobiology of embryos and gametes from laboratory animal species in order to improve long-term preservation of germ plasm.

METHODOLOGY:

Pre-implantation stage embryos were collected from hormonally-treated or natural estrus females following euthanasia. Embryos were frozen or vitrified straws containing medium with cryoprotectant. Straws were stored in liquid nitrogen (-196°C) after which the contents were warmed and the cryoprotectant removed using a sucrose dilution procedure. The viability of diluted embryos was assessed in vitro or in vivo. In vitro evaluation involved development to expanded blastocyst during culture. In vivo evaluation involved surgical transfer of embryos into synchronized recipients. Recipients were allowed to go to term. Individual animal pedigree records for embryos added to the cryobank were maintained on the Genetic Resource Section animal database system.

MAJOR FINDINGS:

Since 1984, approximately 312,084 mouse embryos have been collected. Of these, approximately 107,665 were permanently banked and the remainder were used in comparative biological studies. To date, a minimum of 1,000 non-pedigreed embryos have been stored from each of 35 NIHAGR genotypes. During FY 1995, over 42,989 embryos were collected from 132 mouse strains. All embryos banked this year were collected from pedigreed donors. More than 17,627 embryos were cryopreserved from 120 genotypes and 16,520 of these were permanently banked. The embryo bank now contains approximately 47,393 pedigreed embryos.

Efforts to routinely bank mouse embryos continued this year. With the inclusion of rat embryo donors in the weekly schedule in May 1994, mouse collection days were reduced from 5 per week to 4. In December 1994, rat embryo collections were increased to twice weekly and mice decreased to 3 days. Despite the reduction in collection days devoted to mice, slightly more embryos (16,520) were banked during this fiscal year than last (16,506). This was possible because additional donor mice were included on each day providing an average of 100 mice per week.

Of interest is the variability observed among individual mouse donors of the same genotype. Excluding the genotypes (8%) to-date that have produced no embryos suitable for banking, the proportion of hormonally-treated females, within a genotype,
contributing embryos to the bank varies greatly. As few as 6% (NZW/N-nu*) or as many as 70% (C3H/HeN-bg) of donors yield freezable embryos. Evaluating data from all donor mice to-date suggested that sire, dam and dam parity did not contribute to the response of the donor daughter.

SIGNIFICANCE:

Conservation of valuable germ plasm poses problems that have not generally been addressed as resource management and biomedical research issues. Because of the wide range of species and genotypes representing several genetic backgrounds maintained in the NIHAGR, this is an important issue. Gamete cryopreservation presents a convenient and economical method to maintain the gene pool while limiting the impact of genetic drift on these colonies. Genotype and species differences continue to impact on the efficiency of this is effort.

One of the ongoing concerns of any attempt to bank embryos is the need to maximize the response of the embryo donors. Analysis of the mouse embryo banking data continues to point out the complicating factors contributed by genotype which suggest further refinements may be required to achieve this goal.

PROPOSED COURSE:

Continuation.

PUBLICATIONS:

Schiewe MC, Hollifield VM, Kasbohm LA, Schmidt PM. Embryo importation and cryobanking strategies for laboratory and wildlife species. Proceedings of the annual meeting of the International Embryo Transfer Society. Calgary: Theriogenology, 1995;43:97-104.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-00087-06 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Serum Cholesterol and Triglyceride Values of the NCCR WHHL Rabbit Colony

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis E. Barnard, M.S.	Biologist	LSS, SSB, VRP, NCRR
Donna Jarrell, D.V.M.	Veterinarian	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
Myrna Mandel, Ph.D.	Microbiologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Veterinary Resources Program, National Center for Research Resources, has established a Watanabe heritable hyperlipidemic (WHHL) rabbit breeding colony as a resource for investigators requiring this animal model for use in human familial hypercholesterolemia caused by a low density lipoprotein receptor defect. The purpose of this project is to evaluate the influence of genotype on the serum cholesterol and triglyceride concentrations of the NIH WHHL rabbits. Blood was collected from WHHL rabbits in 1991 and 1994. The 1991 mean total serum cholesterol and triglyceride concentrations were 828 and 394 mg/dl, respectively. Serum cholesterol and triglyceride values ranged from 310 to 1251 mg/dl and 107 to 959 mg/dl, respectively. The 1994 total serum cholesterol values ranged from 222 to 1356 mg/dl with a mean of 572 mg/dl. Serum triglyceride values ranged from 134 to 1410 mg/dl with a mean of 423 mg/dl. The 1994 decrease in total serum cholesterol is due to lower concentrations in WHHL rabbits one year and older (406 mg/dl) compared to similarly aged rabbits in 1991 (550 mg/dl).

OBJECTIVES:

The Veterinary Resources Program, National Center for Research Resources, has established a Watanabe heritable hyperlipidemic (WHHL) rabbit breeding colony as a resource for investigators requiring this animal model for use in human familial hypercholesterolemia due to a low density lipoprotein receptor defect. The purpose of this project is to characterize the serum cholesterol and triglyceride values, and evaluate the influence of genotype on cholesterol and triglyceride concentrations of the NIH WHHL rabbit colony. Analyses for lipoprotein cholesterol and triglyceride concentrations are being reimplemented this year to enhance colony management.

METHODOLOGY:

Blood was collected from WHHL rabbits in 1991 and 1994. The serums were analyzed for lipoprotein cholesterol and triglycerides. Blood will be collected from WHHL rabbits for the next year, and the lipoprotein cholesterol and triglyceride values will be compared with the results from the previous analyses. A computer program has been developed to track pedigrees of the WHHL rabbits, making it possible to evaluate the influence of genotype on cholesterol and triglyceride values.

MAJOR FINDINGS:

The 1991 mean total serum cholesterol and triglyceride concentrations were 828 and 394 mg/dl, respectively. Serum cholesterol and triglyceride values ranged from 310 to 1251 mg/dl and 107 to 959 mg/dl, respectively. The 1994 total serum cholesterol values ranged from 222 to 1356 mg/dl with a mean of 572 mg/dl. Serum triglyceride values ranged from 134 to 1410 mg/dl with a mean of 423 mg/dl. The 1994 decrease in total serum cholesterol is due to lower concentrations in WHHL rabbits one year and older (406 mg/dl) compared to similarly aged rabbits in 1991 (550 mg/dl). The overall colony mean triglyceride concentrations were similar in 1991 and 1994. However, the triglyceride concentration for rabbits one year and older in 1994 was 287 mg/dl compared to 400 mg/dl for similarly aged rabbits in 1991.

SIGNIFICANCE:

The changes in cholesterol and triglyceride concentrations indicate that WHHL rabbits should be monitored for these indices to minimize possible genetic drift.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00090-06 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the WHHL Rabbit Lipoprotein Phenotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis E. Barnard, M.S.	Biologist	LSS, SSB, VRP, NCRR
Myrna Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
Donna Jarrell, D.V.M.	Veterinarian	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
James Crowell, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Veterinary Resources Program, National Center for Research Resources, has established a Watanabe hereditable hyperlipidemic (WHHL) rabbit breeding colony as a resource for investigators. Watanabe et al. reported that there are two types of WHHL rabbits: type 1, those with premature coronary atherosclerosis; and type 2, those with slow development of coronary atherosclerosis. Both types of WHHL rabbits are characterized by high cholesterol and triglyceride concentrations. The two types of WHHL rabbits can be differentiated by their lipoprotein phenotypes.

Agarose gel electrophoresis was used to determine the plasma lipoprotein electrophoretic patterns of 14 rabbits from the NCRR WHHL rabbit colony. The results showed that there are at least two lipoprotein phenotypes in the NCRR WHHL rabbit colony, and one is characterized by beta-VLDL. Findings by other investigators showed the properties of beta-VLDL isolated from WHHL rabbits are similar to those observed in humans with type III hyperlipidemia. The data suggest that there is a population of WHHL rabbits with both an apoprotein E and LDL receptor defect.

Apoprotein E is being isolated from WHHL rabbit VLDL to determine if it is defective. Characterization of apoprotein E from NCRR WHHL rabbits may result in an animal model for type III hyperlipidemia. Identification of the two types of WHHL rabbits will be invaluable to the scientific community because it will eliminate the source of a possible confounding variable when WHHL rabbits are the laboratory animal model.

OBJECTIVES:

The purpose of this study is to characterize the lipoprotein phenotypes in the NCRR WHHL rabbit colony, so that the two types of WHHL rabbits can be identified, and determine if an apoprotein E defect is present in the WHHL rabbits.

METHODOLOGY:

Rabbits are fasted overnight and blood is collected in EDTA vacutainers the next morning. Agarose gel electrophoresis is used to identify the lipoprotein phenotypes. Very low density lipoproteins (VLDL) are isolated by ultracentrifugation. Apoprotein E is isolated from the VLDL and phenotyped by the Western blot method.

MAJOR FINDINGS:

Two lipoprotein phenotypes were identified: one with an increased density of beta-lipoprotein and the other with a beta-doublet. These phenotypes are similar to those reported by other investigators for type 1 and type 2 WHHL rabbits. The triglyceride concentration of the rabbits with the beta-doublet lipoprotein phenotype was significantly greater, 498±62 mg/dl, than that of the other rabbits, 260±65 mg/dl. However, the total serum cholesterol concentrations were not significantly different. The results of this study indicate that the NCRR WHHL rabbits with the beta-doublet phenotype and the Japanese type 1 WHHL rabbit have similar VLDL characteristics.

SIGNIFICANCE:

The two lipoprotein phenotypes indicate that both the WHHL rabbit genotypes (those with the high incidence of coronary atherosclerosis and those with a low incidence) may be present in the colony. Determination of the lipoprotein phenotypes will enable the Genetic Resource Section to selectively breed the rabbits, providing a more defined WHHL rabbit and a new animal model. The cholesterol and triglyceride concentrations and the beta-doublet lipoprotein phenotype observed in NCRR WHHL rabbits as well as the Japanese type 1 WHHL rabbit lipoprotein phenotype and VLDL characteristics are similar to those found in humans with type III hyperlipoproteinemia. Type III hyperlipoproteinemia is the result of an apoprotein E defect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00095-05 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Ophidian Paramyxovirus Infection in Brown Tree Snakes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elaine W. Lamirande, B.S.	Biologist	LSS, SSB, VRP, NCRR
Thomas W. Spencer, B.S.	Microbiologist	LSS, SSB, VRP, NCRR
Jennie W. Owens, M.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Department of Pathology, National Zoological Park, Smithsonian Institution

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH; Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.15	0.05	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is an ongoing project to determine the susceptibility of brown tree snakes (Boiga irregularis) to ophidian paramyxovirus infection and to study the pathogenesis of viral infections with this important group of snake pathogens. Two newly-isolated strains of ophidian paramyxovirus are currently being studied, in addition to further research with a previously isolated virus. Following inoculation with these viruses, brown tree snakes have become clinically ill and up to 50% have died in some inoculation trials. Paramyxoviruses have been isolated from infected snakes and propagated in cell culture and embryonated chicken eggs; the viruses have also been identified via transmission electron microscopy. Snake inoculation trials are continuing at the National Zoo and the Veterinary Resources Program is continuing virus isolation and culture research.

OBJECTIVES:

To experimentally inoculate the brown tree snake (Boiga irregularis) with ophidian paramyxovirus and compare/contrast the pathogenesis of infections with different strains of paramyxovirus in this species of snake.

METHODOLOGY:

Two new viruses were obtained from the University of Tennessee Clinical Virology laboratory. One is a paramyxovirus isolated from the lung tissue of a neotropical rattler (Crotalus durissus), the other is a paramyxovirus isolated from the skin tissue of a reticulated python (Python reticularis). Additional brown tree snakes were inoculated with a paramyxovirus originally isolated from the tissues of an Eastern king snake (Lampropeltis getulus) and then re-isolated from brown tree snakes infected with the virus in a previous inoculation trial.

All snakes were inoculated with the viruses intratracheally and per os. The snakes are individually housed in Horsfall isolation units and are closely monitored for signs of illness. Blood samples are collected periodically and assayed for changes in the hemograms and serum biochemical parameters. Sera is also used for the development of assays to detect host antiviral antibodies. Complete necropsies are performed on any snakes that died and tissue samples are collected to histopathology, virus isolation, and electron microscopy.

MAJOR FINDINGS:

The different strains of paramyxovirus have demonstrated varying cytopathic effects in cell culture and have induced varying degrees of respiratory disease in the inoculated brown tree snakes. Paramyxoviruses have been isolated from brown tree snake tissues through cell culture and embryonated chicken eggs and identified by transmission electron microscopy.

SIGNIFICANCE:

Snakes and other reptiles are commonly kept in captivity, and their use as laboratory animals is increasing. Relatively little is known about the diseases of these animals. This study represents the first known attempts to experimentally transmit ophidian paramyxovirus in brown tree snakes. Two strains of paramyxovirus have caused significant disease in brown tree snakes, suggesting that further studies with these and/or similar viruses are warranted and could discover potential biologic control agents for this snake species.

PROPOSED COURSE:

Continuation.

PUBLICATIONS:

Nichols DK, Lamirande EW. Use of methohexital sodium as an anesthetic in two species of colubrid snakes, Proceedings of the American Association of Zoo Veterinarians 1994;161-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10400-04 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Idiopathic Hepatitis in the N:NIH(S)-B Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jennifer A. Burris, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Michael A. Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Dennis E. Bernard, M.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, LSS

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.02	0.01	0.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

All N:NIH(S)-B mice have multiple foci of hepatic necrosis and inflammation. The cause of hepatitis is unknown. This mouse strain was developed at the NIH Genetic Resource for use as foster mothers in a barrier facility. The mice appear clinically normal, but have macroscopic, microscopic, and biochemical liver abnormalities. Studies were conducted to determine the etiology of hepatitis, to rule out the presence of an infectious agent, and to explore the pathogenesis of hepatitis in this strain. High levels of dietary vitamin A was examined as a possible etiology or co-factor. Closely related mouse strains, the N:NIH(S) and the CR:NIH(S), were examined for the hepatic abnormalities seen in the N:NIH(S)-B.

OBJECTIVES:

To determine the etiology and study the pathogenesis of idiopathic mouse hepatitis. To rule out the presence of an infectious agent causing hepatitis.

METHODOLOGY:

Mice were housed in a conventional facility in filter top cages. They were autopsied at 9, 10, and 12 weeks and at 12, 18, and 24 months of age. Gross and histologic lesions were assessed. SCID mice were orally and parenterally inoculated with fresh homogenized affected livers and then were autopsied at 1, 2, and 3 weeks post-inoculation. Serology, TEM, serum chemistries, liver cultures (aerobic, anaerobic, routine, and special for Helicobacter), special histologic stains, and immunocytochemistry were done. Mice on three special diets (low vitamin A, normal vitamin A, and high vitamin A) were paired and progeny examined at 10 weeks of age for lesions.

MAJOR FINDINGS:

Chronic and active hepatitis was detected in all ages examined from 2 weeks to 2 years of age. There were a range of microscopic lesions, many of which were found in any individual mouse, and all of which were found within any age group. The lesions were most severe and extensive in 12 week and 12 month old mice, however serum enzymes which indicate liver disease were rarely and only mildly elevated. Most mice allowed to age to 24 months survived and remained healthy, and those that died prior reaching this time point died of lymphoma. An infectious etiology was ruled out based on a variety of examinations and experiments to detect an agent including serology, bacteriology, transmission electron microscopy, light microscopy with special stains, immunocytochemistry, and transmission studies. Infection with the recently discovered murine hepatic Helicobacter sp. was ruled out. Results indicate that high levels of vitamin A in the diet reduces the incidence and severity of hepatitis. The closely related N:NIH Swiss mouse has a comparable high incidence and severity of idiopathic hepatitis; however, closely related CR:NIH Swiss mice have a low incidence and severity. This suggests a possible role for environmental or genetic factors in the pathogenesis of the disease. Idiopathic hepatitis has also been detected in numerous other strains of mouse at a very low incidence and severity.

SIGNIFICANCE:

The etiology of hepatitis in these mice remains unknown, i.e. idiopathic. An infectious etiology appears highly unlikely. Environmental or genetic factors may contribute to the disease. Dietary vitamin A levels, often high in commercial feeds, do not cause or worsen the disease but instead at high levels may protect against liver damage. This disease has been detected in numerous strains of mice. Affected mice appear clinically normal and live normal life spans, therefore the impact this disease may have on research using mice remains uncertain.

PROPOSED COURSE:

Termination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-RR-10432-03 VR

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenicity of CAR Bacillus (mouse origin) in Immunodeficient Strains of Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Thomas Spencer, B.S.	Microbiologist	LSS, SSB, VRP, NCRR
Elaine Lamirande, B.S.	Biologist	LSS, SSB, VRP, NCRR
Annie Merriweather, B.S.	Histotechnologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Veterinary Resources Program

SECTION
Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION
NCRR, NIH, Bethesda Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.05	0.05

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was undertaken to determine the extent and severity of respiratory disease caused by CAR bacillus in immunodeficient mouse strains. Mice were inoculated intranasally with a standard number of CAR bacilli. These mice were killed at 2, 4, 8, and 16 weeks post inoculation. Tissues were obtained to determine the extent and severity of lesions by histopathological examination. The in-vivo portion of the pathogenesis study has been completed and histopathologic analyses demonstrated that pulmonary lesions were most severe in BALB/c, comparable in severity in the CB17 scid and nu/nu, and least severe in the bg-nu-xid. An additional study using inoculated and naive BALB/c mice was completed which demonstrated the ability to transmit the agent via an aerosol route. The initial transmission study has been completed and histopathologic analyses are in progress.

OBJECTIVES:

To evaluate the extent and severity of respiratory disease caused by CAR bacillus in several common immunodeficient mouse strains used in research worldwide, and to investigate the transmissibility of this agent by an aerosol route.

METHODOLOGY:

Pathogenesis Study

A total of 25 mice each of the following strains: BALB/c, CB17 scid, nu/nu and bg-nu-xid mice were inoculated intranasally with 0.02 ml of allantoic fluid containing a standardized number of CAR bacillus organisms. Five mice per strain were inoculated with normal allantoic fluid as negative controls. Five inoculated mice and one negative control mouse per strain were sacrificed at the following time points: 2 weeks, 4 weeks, 8 weeks, and 16 weeks. Immunocompetent mice were anesthetized with metofane and bled to collect serum prior to euthanasia. Tracheal washes were performed at sacrifice on all animals and examined for the presence/absence of CAR bacillus. The trachea, lungs, and nasal turbinates were collected and fixed in 10% neutral buffered formalin for histologic evaluation.

Transmission Study

Ninety five-week old female BALB/c mice were maintained in polycarbonate cages with wire lids in Horsfal units, having filtered, unidirectional air flow. Forty five of the mice were inoculated intranasally with 2×10^6 CAR bacilli. Cages containing aerosol exposed mice were positioned directly behind cages containing inoculated mice. The mice were periorbitally bled at monthly intervals for 6 months, beginning at 8 weeks post inoculation. At 6 months the mice were sacrificed, necropsied, and sections of the respiratory tract were collected for histopathology. Tracheal washes were performed and sera was collected for analysis.

MAJOR FINDINGS:

Pathogenesis Study: Mice inoculated with CAR bacillus developed bronchopneumonia, bronchiectasis, and otitis media. The lesions were most severe in the BALB/c mice, comparable in severity in the CB17 scid and nu/nu, and least severe in the bg-nu-xid mice.

Transmission study: Serologic, tracheal wash, and histopathologic analyses confirmed aerosol transmission of the bacillus and infection of BALB/c mice housed in four of the nine cages.

SIGNIFICANCE:

The results of the pathogenesis study indicate that while immunocompromised mice develop significant lesions due to infection with CAR bacillus, a competent immune system contributes to the overall severity of disease. The transmission study confirms the potential of aerosol transmission from one cage to another.

PROPOSED COURSE:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10433-03 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection and Diagnosis of Mycobacterial Infection in Nonhuman Primate Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ali A. Azadegan, D.V.M., Ph.D.	Clinical Veterinarian/ Microbiologist	GRS, SSB, VRP, NCRR
Myrna G. Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
Toni O'Neill, B.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Tulane Regional Primate Research Center

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polymerase chain reaction (PCR) is a rapid, reproducible and reliable method of detecting specific sequences of DNA. The sensitivity of PCR to various strains of mycobacterium depends on the degree to which the target sequence is conserved in the organism. The specificity depends on the extent to which the target sequence is unique to the organism. The objective of the study is to develop a rapid and specific laboratory test for detecting and identifying mycobacteria within clinical samples from nonhuman primates. We have used published genus-specific primers to synthesize probes in order to detect and differentiate the major pathogenic mycobacterial species. This was done in order to amplify the dnaJ genes from a broad spectrum of mycobacterial species. We and investigators at Tulane Regional Primate Research Center (Tulane RPRC) have analyzed two sets of blind coded bronchoalveolar lavage, stomach lavage, urine and rectal swab samples for mycobacterial antigens. These samples were obtained from the group of NIH nonhuman primates housed at the Tulane Regional Primate Research Center (Tulane RPRC). The two sets of samples were obtained from the animals at an interval of 6 months apart. The Tulane RPRC investigators used different mycobacterial probes. Upon analysis of the data from the two sets of blind coded samples, both groups identified a mycobacterial antigen in the same animals in each sampling period. However, use of both sets of probes in group of animals tested did not indicate a significant improvement in the diagnosis of nonhuman primates tuberculosis.

OBJECTIVES:

To develop a rapid, reproducible, reliable and specific laboratory test for detecting and identifying mycobacterial infections in nonhuman primates.

METHODOLOGY:

Bronchoalveolar lavage, stomach lavage, blood, urine and rectal swabs were obtained from ketamine HCL anaesthetized animals. These were treated appropriately resulting in a suspension from which genomic DNA will be isolated utilizing sodium dodecyl sulfate (SDS), ribonuclease A and proteinase K followed by phenol-chloroform extraction and ethanol precipitation. DNA was amplified in a thermocycler according to standard procedures. PCR primers were prepared based on the nucleotide sequence of the dnaJ gene of *M. tuberculosis.* The labeled species-specific oligonucleotide sequences synthesized to probe PCR products were prepared from four mycobacterial species on the basis of the individual dnaJ genes sequences differing by more than two bases from each other. Dot blot analyses was used to evaluate the amplified dnaJ gene products from the four mycobacterial species and from clinical isolates by hybridizing samples with the labeled species-specific oligonucleotide probes. Resulting autoradiograms will provided permanent records of relatedness between different species and confirmation of the preliminary diagnosis.

MAJOR FINDINGS:

We and investigators at Tulane Regional Primate Research Center (Tulane RPRC) analyzed two sets of blind coded bronchoalveolar lavage, stomach lavage, urine and rectal swab samples for mycobacterial antigens. These samples were obtained from the group of NIH nonhuman primates housed at the Tulane Regional Primate Research Center (Tulane RPRC). The two sets of samples were obtained from the animals at an interval of about 6 months apart. The Tulane RPRC investigators used a different mycobacterial probe. Upon analysis of the data from the two sets of blind coded samples, both groups identified a mycobacterial antigen in the same animals in each sampling period. However, using our probes did not improve overall diagnosis of tuberculosis over traditional bacterial culture.

SIGNIFICANCE:

Tuberculosis remains one of the most important zoonotic diseases of man and primates. Despite the constant effort to test and identify potentially exposed and infected primates in laboratory animal facilities, a percentage of infected animals are diagnosed in necropsy rooms. Definitive diagnosis of tuberculosis infections is provided only by demonstration of the presence of mycobacteria in clinical specimens. Microscopic examination which has been the basic method for the diagnosis of tuberculosis requires a relatively large number of bacteria to be present in specimens. Culture confirmation is a sensitive method for detecting mycobacteria, but it takes 3 to 8 weeks and another 2 to 4 weeks for final identification by biochemical analyses. The results of the study indicate that the polymerase chain reaction with genus-specific primers that amplify the dnaJ genes and subsequent dot blot analysis with species-specific oligonucleotide probes may be useful for diagnosis of primate mycobacterial infection.

PROPOSED COURSE:

Complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10434-03 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Embryo Cryopreservation Procedures for Laboratory Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William F. Rall, Ph.D	NRC Senior Research Associate	GRS, SSB, VRP, NCRR
Patricia M. Schmidt, M.S.	Physiologist	GRS, SSB, VRP, NCRR
Xi Lin, D.D.S.	Visiting Scientist	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH Animal Genetic Resource (NIHAGR) maintains many unique rat genotypes used as models of normal and disease processes in man. Effective management of this resource would be facilitated by the development of embryo cryopreservation procedures for long-term banking. In order to develop banks of cryopreserved germ plasm from laboratory rats, protocols for each step of the embryo banking and rederivation process must be established. These would include the preparation and mating of embryo donor females, embryo collection, cryopreservation, thawing, and embryo transfer. Previous studies suggest that superovulation procedures developed for laboratory mice are not as effective when applied to laboratory rats. The principal objective of this project is to evaluate treatment with an analog of gonadotropin releasing hormone (GnRH-a) as an alternative approach to synchronize estrus cycles for embryo collection and cryopreservation.

OBJECTIVE:

The objectives of this study are to: 1) evaluate the efficacy of gonadotropin releasing hormone analogue (GnRH-a) for synchronizing the estrus cycle of adult female rats from selected NIH Animal Genetic Resource (NIHAGR) genotypes; 2) collect and cryopreserve embryos from treated and mated females; and 3) determine the viability of cryopreserved rat embryos.

METHODOLOGY:

Adult female rats from selected NIHAGR genotypes were injected with a gonadotropin releasing hormone analogue (GnRH-a) and paired 2 to 4 days later with a fertile male. Successful mating was confirmed the morning 5 days after GnRH-a treatment by the presence of spermatozoa in vaginal washes. (Mating was not confirmed when females were paired barrier facilities). Preimplantation stage embryos were collected from euthanized females 5 days after sperm was detected or, in the case of females maintained in barrier facilities, 9 days following injection of GnRH-a. Embryos were cryopreserved by either conventional slow freezing or vitrification methods using glycerol as the cryoprotectant. Following storage in liquid nitrogen (-196°C), embryos were warmed and diluted out of the cryoprotectant solution. The viability of embryos was determined by continued development either in vitro to expanded blastocysts or in vivo to normal pups. Individual animal pedigree records were maintained on the Genetic Resource Section animal database system for embryos cryopreserved for long-term banking.

MAJOR FINDINGS:

Since 1992, approximately 11,014 pedigreed rat embryos have been collected. Of these approximately 10,132 embryos were permanently banked and the remainder used for quality control assays and developmental research to optimize cryobiological procedures. During FY 1995, approximately 9,581 embryos were collected from 101 rat genotypes. Most (9,023) were banked for long-term storage. To date, at least 500 embryos were banked from four genotypes, two of which now exist only as cryopreserved embryos. The genotype of donor females was found to affect the efficiency of embryo banking in two ways. Analysis of those genotypes (n = 25) with at least 20 treated females indicates: 1) the proportion yielding freezable embryos (mean = 65%) varied considerably among genotypes (range: 14-94%); and 2) the mean number of embryos from females yielding embryos (7.8) also varied considerably among genotypes (range: 2.8-10.3). These data indicate that based on 25 rat genotypes, an average of 93 females (range 58-250) must be treated to obtain 500 embryos for cryobanking. Preliminary analysis suggests that low fertility of males and females, behavioral incompatibilities or anatomical abnormalities (e.g., ipsilateral gonadal agenesis) may account for differences in embryo production for some genotypes. Modifications of methods used to prepare donor females may increase the efficiency of embryo production from these genotypes. The viability of banked embryos was confirmed by in vitro culture and embryo transfer. Most (85%, n = 125) of the thawed embryos developed to expanded blastocysts. A total of 150 thawed embryos from 9 genotypes was transferred into the uterine horns of foster mothers. All recipients maintained pregnancy and 35% of the thawed embryos developed into normal pups. Pups derived from banked embryos had normal fertility.

SIGNIFICANCE:

The synchronization of estrus cycles of rats using GnRH-a provides a convenient method for increasing the efficiency of embryo collection and cesarian rederivation for rat genotypes maintained by the NIHAGR. Embryo cryopreservation studies indicated that the standard slow freezing and vitrification procedures developed for mouse embryos are equally effective for rat embryos. The banking of pedigreed embryos from females treated in a manner analogous to that currently employed to prepare mice for embryo cryopreservation indicates the suitability of this approach.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10435-03 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A New Pathogenic Bacterium of the Haemophilus/Pasteurella Group in Hamsters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jennifer A. Burris, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Michael A. Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Georgina F. Miller, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Elaine Woo, B.S.	Biologist	LSS, SSB, VRP, NCRR
Theresa Lawrence, Ph.D.	Microbiologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.02	0.01	0.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed tha space provided.)*

An unclassified member of the bacterial family Pasteurellaceae has been isolated from cases of enteritis, conjunctivitis, and abscessation in a colony of golden hamsters (Mesocricetus auratus). This bacterium is a member of the Haemophilus/Pasteurella group, based on routine biochemical testing. It may represent a new species, but additional studies such as DNA hybridization may help to define the taxonomic position.

Three golden hamster strains are maintained in the NIH Animal Genetic Resource - N:Syr (Syrian), N:M70 (Murphy), and N:LSH/TrN. The Haemophilus/Pasteurella group bacterium has been isolated from all strains. Both clinically ill and healthy adult hamsters representing all strains have been examined for gross and microscopic abnormalities, identification of gastrointestinal parasites, characterization of intestinal microflora, and identification of pathogenic bacteria at sites of inflammation.

Syrian hamsters from outside the colony have been used to determine the relative pathogenicity of the Haemophilus/Pasteurella group bacterium. Various routes of inoculation and doses of bacteria have been combined with clinical observations and post-mortem studies. This unique bacterial species in the Haemophilus/Pasteurella group is widespread in the hamster colony and may be responsible for significant morbidity and mortality in this colony. This study will help define the lesions produced, extent of the disease in the colony, and will result in the development of appropriate techniques to eliminate this infection from the colony and thus improve the health of animals used by NIH scientists.

OBJECTIVES:

To test the pathogenicity of the Haemophilus/Pasteurella group bacterium, to determine the extent of natural infection in the colony, to classify this bacterium, and to identify spontaneous diseases associated with this bacterium in the colony.

METHODOLOGY:

Hamsters were housed in conventional facilities in open-top cages. Complete autopsies were performed, tissues fixed in neutral buffered formalin and routinely processed for light microscopic examination. Standard culture and sensitivity techniques were employed. Isolation of the Haemophilus/Pasteurella group bacterium was enhanced by GN broth and blood agar in a CO_2 atmosphere. Bacterial suspensions for ocular, oral, subcutaneous, and intramuscular injections were standardized using the McFarland nephelometer technique. Bacterial suspensions for experimental injections were obtained from frozen subcultures originally from clinical material.

MAJOR FINDINGS:

The Haemophilus/Pasteurella group bacterium was common in the intestinal tract of all three strains of hamsters in the colony, and was often the predominant flora. Coliform bacteria, though the predominant intestinal flora in CR:Syr hamsters from outside the colony, were absent from the three colony hamster strains. The H/P group bacterium was characterized by routine biochemical tests and placed into the Haemophilus/Pastuerella group, but additional characterization to accurately classify this bacterium was not done. Occasional biochemical variants of the Haemophilus/Pasteurella group bacterium were found. This bacterium was associated with numerous spontaneous cases of enteritis, conjunctivitis, metritis, pneumonia, wounds, and abscesses in the hamster colony. Sensitivity testing of the bacterium lead to the selection of an antibiotic used to treat the entire colony which lead to a drastic reduction in colony losses. Oral and parenteral administration of this bacterium to immunocompetent young adult CR:Syr hamsters did not cause gut colonization or disease. This bacterium therefore appears to be opportunistic and may require other co-pathogens to produce infections. For example in the gastrointestinal tract, heavy infections with Giardia were associated with enteritis and overgrowth of the Haemophilus/Pasteurella group bacteria. In wounds and abscesses, other pathogenic bacteria were detected along with the Haemophilus/Pasteurella group bacterium.

SIGNIFICANCE:

This unclassified bacterial species in the Haemophilus/Pasteurella group was widespread in the NIH Animal Genetic Resource hamster colony and was associated with disease processes which lead to significant colony losses. Following specific antibiotic treatment, losses were greatly reduced. Pathogenicity testing indicated that this bacterium was an opportunistic rather than a primary pathogen.

PROPOSED COURSE:

Termination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10436-03 VR

PERIOD COVERED

October 1,1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Fingerprinting Rodents in the NIH Repository

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Myrna G. Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
James S. Crowell, Jr., Ph.D.	Geneticist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Science Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

After two years and more than 1,000 tissue homogenates, we are beginning to quantitate our computer database of profile information in order to further augment our genetic quality assurance program. We can now supplement raw data with sophisticated densitometry of our autoradiograms and gels. Though we are still using biochemical isozymes to routinely check the genetic authenticity of rodents used as experimental models both within and outside NIH, we now regularly supplement these assays with DNA fingerprinting. We have found the technique absolutely essential in testing for genetic purity in both recombinant and congenic strains. It is perhaps the only reliable way to distinguish between certain species of rodents.

OBJECTIVES:

To develop DNA fingerprinting as an effective and efficient method for use by genetic quality assurance programs.

METHODOLOGY:

Small pieces of tails and livers have been collected and homogenized from the various inbred, congenic, and recombinant strains of rodents that have been received for genetic monitoring. Following incubation with a detergent (SDS, sodium dodecyl sulfate) and a protease (proteinase K), the homogenized and degraded tissues were extracted several times with phenol/chloroform, incubated with ribonuclease (to degrade ribonucleic acid) and finally were precipitated in isopropanol. Purified DNA was then incubated with a restriction endonuclease that cut DNA fragments in a unique pattern, depending on where and how often a particular repetitive sequence, recognized by the enzyme, occurred in the strain's DNA. The DNA digests were then run very slowly (to increase resolution) on large agarose gels, transferred to nylon via Southern blotting, and exposed, under appropriate hybridization conditions, to a multilocus, alkaline phosphatase labelled probe that reacts with a chemiluminescent substrate producing a transient intermediate that degrades to yield a signal on a photographic film. If the probe exposed to the unknown DNA finds sequences to complement, it will hybridize (attach itself) forming a fingerprint on the film. Additional studies had been initiated to determine if radioactively labelled, double stranded probes would give more reproducibility. Since these probes did indeed yield superior data, we have modified our probe protocol, substituting a double stranded probe labelled radioactively via primer extension for the multilocus probe previously used.

MAJOR FINDINGS:

This past year we have found aberrant bands on DNA fingerprints apparently due to mutations or contamination of a variety of rodent models sent to us. We are continuing to search for the cause of unusual bands, for example, in a group of C57BL/6 and NFR mice, both of which appeared genetically pure biochemically, but not on fingerprints. The former strain had yielded anomalous skin graft results, while the latter displayed other unexplained characteristics. We can now also use DNA fingerprinting to distinguish between two commonly used strains of guinea pigs and are the only facility to offer this service. We have nearly completed the DNA fingerprinting of the entire albino rat strains in the NIH Animal Genetic Resource (NIHAGR). Since this morphologically indistinguishable group is difficult to differentiate by more conventional methods as well, this work provides another important quality assurance tool. The increased use of embryo cryopreservation technology by the NIHAGR has resulted in an increased demand for insuring the authenticity of rederived animals.

SIGNIFICANCE:

DNA fingerprinting can be used to test the genetic purity of congenic and recombinant strains (as well as inbred ones). No other genetic monitoring technique can do this with confidence. Additionally, in contrast to most other techniques, DNA fingerprinting allows an investigator to submit a tail snip for testing, thus obviating the need to sacrifice an important research animal.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10437-03 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infection with Mycoplasma pulmonis in SCID and BALB/C Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Georgina F. Miller, D.V.M	Staff Veterinary Pathologist	LSS, SSB, VRP, NCRR
Patrick Guinn, B.S.	Biologist	LSS, SSB, VRP, NCRR
David Madden, D.V.M.	Chief, LSS	LSS, SSB, VRP, NCRR
Myrna Mandel, Ph.D.	Microbiologist, GMU	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Diagnostic Laboratory Unit & Genetic Monitoring Unit, VRP

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mycoplasma pulmonis is one of the most important pathogens of laboratory rodents. It is the etiologic agent of Murine Chronic Respiratory Disease (CRD) which was ubiquitous in laboratory rats and mice into the 1970's. This disease has almost been eliminated. In research colonies, detection of subclinical infections by parasitic, bacterial, viral, and mycoplasmal agents generally depends on testing of designated sentinel animals. Because immunodeficient mice do not make antibodies, serologic tests, which would detect them, are of no value in these animals. However, the nearly universal practice of housing immunodeficient mice in microisolator cages effectively isolates the sentinels (who may be housed in the same rack but in different cages) from any exposure to pathogens harbored by the immunodeficient mice. The objective of this study is to 1) determine the gross and histopathologic changes produced by Mycoplasma pulmonis in immunodeficient mice (SCID) inoculated by both the respiratory and genital routes and compare them with those found in immunocompetent mice (BALB/C) under identical conditions; and 2) establish the reliability of polymerase chain reaction (PCR) techniques in detecting mycoplasmosis, and compare its sensitivity with that of routine culture. Mice will be infected by either the intranasal or intravaginal route. Infection will be detected by serology (as appropriate), culture, PCR, and by gross and histologic changes. Samples for mycoplasma culture and PCR will be collected from the oropharynx, vagina, and uterus. The following tissues will be screened for histologic changes: nasal sinuses, middle ears, trachea, lungs, vagina and uterus.

PHS 6040 (Rev. 5/92)

OBJECTIVE:

1. Determine the gross and histopathologic changes produced by *Mycoplasma pulmonis* in immunodeficient mice (SCID) inoculated by both the respiratory and genital routes and compare them with those found in immunocompetent mice (BALB/C) under identical conditions.
2. Establish the reliability of polymerase chain reaction (PCR) techniques in detecting mycoplasmosis, and compare its sensitivity with that of routine culture.
3. Compare the sensitivity of vaginal swab with culture & PCR testing of uterus tissue.

METHODOLOGY:

SCID & BALB/C mice were infected with *Mycoplasma pulmonis* via respiratory and genital routes, and samples for Mycoplasma culture, PCR, and histopathology were collected at 3 timepoints. No difference in sensitivity or histopathologic lesions were found between the SCID and the BALB/C mice. 100% spread of the intravaginal infection to the oropharynx was found. Infection was verified in only a few mice administered the most dilute inoculum. The protocol was modified as follows: only BALB/C mice were employed, all mice were inoculated via the intravaginal route, and different PCR primers were utilized.

MAJOR FINDINGS:

1. No differences in sensitivity or pathologic response to *M. pulmonis* infection by BALB/C and SCID mice were found.
2. 100% of mice infected by the intravaginal route developed colonization of the oropharynx; less than 100% of the mice infected by intranasal inoculation developed genital infection.
3. Mycoplasma culture of oropharyngeal swabs was found to be the most reliable methodology. The PCR methodology employed proved to be less sensitive than Mycoplasma culture. Infected BALB/C mice began to seroconvert by 4 weeks, but even after 4 weeks, serology was not as reliable as culture in detecting infected animals.

SIGNIFICANCE:

The finding that the current PCR methodology is less reliable than culture highlights the need to employ other probes, refine PCR conditions and to verify results with culture.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10442-02 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatic Hemosiderosis in Marmosets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Georgina F. Miller, D.V.M.	Staff Veterinary Pathologist	LSS, SSB, VRP, NCRR
Dennis Barnard, M.S.	Nutritionist	LSS, SSB, VRP, NCRR
Ruth Woodward, D.V.M.	Clinical Veterinarian	PUP, RAB, VRP, NCRR
Bernard Flynn, D.V.M.	Chief, Primate Unit/Poolesville	PUP, RAB, VRP, NCRR

COOPERATING UNITS *(if any)*

Behavior and Nutrition Unit, LSS, SSB and Primate Unit/Poolesville, PS, RAB

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic hemosiderosis is a commonly recognized finding in marmosets as well as other new world monkeys kept in zoologic, breeding and research colonies. Based on the absence of associated liver pathology or elevation in serum liver enzymes in affected animals, the condition has been considered to be of no clinical or pathologic significance. A high incidence of hepatic and systemic hemosiderosis was verified in a retrospective study of marmosets from NIH's colony, who were submitted for routine necropsy to the Pathology unit, LSS, SSB, VRP, NCRR (project # Z01-RR-10438-01 VR). These animals had been used in behavior research and had died or were euthanized due to spontaneous disease or trauma. There was no history of experimental diet or drug administration in these animals. At death, many of the adults were severely wasted due to undetermined causes. In human hereditary hemochromatosis, the most common inherited disease in Caucasians of northern European descent, iron overload causes debility and organ failure, and untreated, can result in premature death. This study was undertaken to determine if hepatic hemosiderosis in marmosets is caused by excess dietary iron, if it results in debility, if it can be prevented by restricting dietary iron, and if it may be a useful model of human iron overload diseases.

OBJECTIVE:

To establish the role of dietary iron in the development of hepatic hemosiderosis in the common marmoset, *Callithrix jacchus*; to document the health affects of this condition as well as possible health benefits of a low iron diet; to assess the ability of blood chemistries to diagnose iron overload; and to establish baseline data which will assist in assessing marmoset hemosiderosis as a model for human iron overload diseases.

METHODOLOGY:

A dedicated colony of young adult common marmosets has been divided into 2 groups, and is being fed nutritionally complete diets which differ only in their iron content. The low dietary iron group receives a diet containing less than 100 ppm iron, while the high dietary iron group receives a diet containing 350 to 500 ppm iron (the iron content of the high iron diet was changed from 500 to 350 ppm following the death of a marmoset receiving the 500 ppm diet; his liver iron content had increased 2.5 fold, to 13,693 ug/g, in 7 months). Baseline blood work, including serum iron and transferrin levels, and liver biopsies for iron analysis were performed prior to administering the test diets. Hematology and serum chemistries are performed every 6 months, with liver biopsies and iron analysis performed every 12 months. Animals are weighed and assessed for health status at regular intervals. A complete necropsy is performed on any animal dying during the study.

MAJOR FINDINGS:

1. 100% of the marmosets on the high iron diet increased the quantity of stored iron in the liver, while 83% (5/6) of the marmosets on the low iron diet had decreased quantity of stored iron.
2. Rate of liver iron accumulation varied markedly among the marmosets on the high iron diet, and may be genetically influenced. The fact that the 2 marmosets with the highest level of iron accumulation were siblings is consistent with genetic predisposition.
3. Rapid accumulation of iron in the liver was associated with premature death. Four of the seven marmosets fed the high iron diet accumulated greater than 10,000 ug/g iron in the liver in less than a year and experienced 100% mortality. The surviving marmosets in the high iron group accumulated less than 6,000 ug/g iron in the liver after 12 months on the diet (representing a 1.7 to 3 fold increase).
4. Marmosets fed a diet containing less than the NRC's recommended iron levels maintained normal hematopoietic activity and experience no clinical evidence of iron deficiency, while accumulating significantly less hepatic iron than marmosets fed a high iron diet. Of the 7 marmosets on the low iron diet, 1 had slightly increased liver iron content, (from 2245 to 3872 ug/g), while the others had decreased liver iron content. The low iron group experienced significantly lower mortality than the high iron group (14% vs 57%).

SIGNIFICANCE:

1. Hepatic iron overload (2 to over 8 fold increase in liver iron content in less than a year) can be consistently achieved in marmosets by feeding a diet high in iron. Iron accumulates within hepatocytes, similar to liver iron deposition of human hereditary hemochromatosis. This suggests that dietary manipulation in this species may provide a useful animal model for human iron overload.
2. The marked variation in liver iron accumulation among the marmosets in the high iron group suggests that factor(s), possibly genetic, may influence the rate of iron accumulation. Genetic screening of individuals who exhibit rapid iron accumulation may lead to selective breeding of a model for human hereditary hemochromatosis.
3. The association of high iron diet, rapid accumulation of iron within the liver, and premature death strongly supports the hypothesis that high liver iron content has negative health implications.
4. Liver iron overload in marmosets can be prevented or curtailed by restricting the iron content of the diet. Dietary modifications suggested by this study, if employed in other marmoset colonies, may prevent development of hemosiderosis and result in healthier animals.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-10468-01 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less Title must fit on one lina between the borders.)*

Evaluation of Commercially Available Bioassays for Detection of Giardia in Rodents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ali A. Azadegan, D.V.M., Ph.D.	Facility Veterinarian/ Microbiologist	LSS, SSB, VRP, NCRR
Thomas H. Spencer, B.S.	Microbiologist	LSS, SSB, VRP, NCRR
David L. Madden, D.V.M.	Chief, LSS	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Giardia species are a group of endoparasites that inhabit the intestinal tract and may cause clinical or subclinical infections in mammals. Detection and identification of Giardia spp. by light microscopy can be difficult or unreliable due to the sporadic shedding of cysts, inadequate number of organisms, improper specimen collection, or the interference of anti-diarrheal medication. Commercial kits utilizing ELISA or immunofluorescent antibody (FA) technologies have recently become available for the diagnosis of human giardiasis. We report here data on the efficacy of two such kits in detecting Giardia infections in hamsters and mice. Animals known to be infected with Giardia spp. as determined by fecal/cecal microscopic examinations were tested utilizing ELISA or FA serological kits. Both kits yielded positive results with hamsters but neither kit was able to detect Giardia muris in infected mice. Substitution of a specific anti-Giardia muris in the FA kit yielded positive results in mice and hamsters but was negative for Giardia lamblia from human samples. Thus, ELISA and FA assays could be a valuable adjunct to the examination for intestinal giardiasis as they can be performed quickly using non-infective formalin fixed fecal material. The detection of false negative results in the present study, however, indicates the importance of determining the exact specificity of the antisera used in the assay.

OBJECTIVES:

To identify *Giardia* strain(s) that infect laboratory animals and to evaluate the commercially available serodiagnostic tests for laboratory animal giardiasis.

METHODOLOGY:

Two serodiagnostic kits, ProSpecT/*Giardia* ELISA (Alexon, Inc.) and MerIFluor direct immunofluorescent detection kit (FA) for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts in fecal material (Meridian Diagnostics, Inc.) were used according to the manufacturer direction. Fecal/cecal samples of mice and hamsters obtained from various sources were collected and suspended in saline or 10% formalin before use.

MAJOR FINDINGS:

Both kits yielded positive results with hamster samples but neither kit was able to detect *Giardia muris* in infected mice samples. These results indicate that *Giardia* strain in hamster is closely related to *G. lamblia* that infect human beings and *G. muris* can be diagnosed when specific anti-*Giardia* monoclonal antibody is substituted in the serodiagnostic kit.

SIGNIFICANCE:

Giardia spp. is an ubiquitous intestinal parasite and frequent cause of gastroenteritis infection in humans and animals. Detection and identification of *Giardia* in stool specimens by conventional microscopy can be tedious and time consuming especially in light infections. The rapid detection of *Giardia* antigens in stool specimens by sensitive, specific and inexpensive methods can alleviate this problem and eliminate the need for complete ova and parasite microscopic examination. Also, our results indicate that ELISA and FA assays could be a valuable adjunct to the examination for intestinal giardiasis. In addition, strain specific monoclonal antibodies should be included in serodiagnostic kits to detect all *Giardia* strains to reduce false positive results.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10469-01 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Helicobacter hepaticus Infection in Laboratory Rod Rodents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ali A. Azadegan, D.V.M., Ph.D.	Facility Veterinarian/ Microbiologist	LSS, SSB, VRP, NCRR
Michael A. Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
David L. Madden, D.V.M.	Chief, LSS	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Diagnostic Laboratory Unit and Pathology Unit, LSS, VRP

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Helicobacter hepaticus is a recently identified species of a gram-negative, microaerophilic, urease-positive, spiral bacterium. Helicobacter hepaticus have been found to cause a chronic active hepatitis in some strains of mice but only enteric infection without clinical or histological disease in other mouse strains. Of the other Helicobacter spp. of mice, H. rappini has not been associated with lesions in mice, H. muridarum has been observed in gastric glands of mice with chronic gastritis, and H. bilis was isolated from older mice and based on 16s rRNA gene sequence analysis was named as a new organism. This new Helicobacter like H. hepaticus colonizes the bile, liver and intestine of the mice. Using the present bacterial culture technique we have been able to isolate Helicobacter hepaticus from A/JCr, DBA/2 Ncr, and C57B/6 strain of mice and a rat strain. Also, by substituting the antimouse conjugate in an ELISA test kit which is prepared to diagnose H. pylori infection in human we have been able to detect antibody to Helicobacter in mice.

OBJECTIVE:

To investigate the transmission and diagnosis of Helicobacter infections in laboratory animals and to apply these findings to the intramural NIH animal facilities.

METHODOLOGY:

Fecal/cecal samples were collected aseptically from donor animals and diluted in sterile saline. Filtered supernatant from this suspension was cultured on Brucella agar plates containing horse blood and TVP (trimethoprin, vancomycin, polymyxin). Plates were incubated in a non-inverted position at 37C under microaerophilic environment, 88% nitrogen, 7%hydrogen and 5% carbon-dioxide for seven days in a modified anaerobic chamber.

MAJOR FINDINGS:

We have been able to culture Helicobacter organisms from several strains of mice (A/JCr, DBA/2Ncr, C57B/6) and one strain of rat. Also by substituting an antimouse conjugate in an ELISA Kit prepared for human Helicobacter pylori, we were able to detect Helicobacter antibody in mice.

SIGNIFICANCE:

The presence of Helicobacter hepaticus in the liver of mice and associated liver lesion has raised questions regarding the use of these mice in biomedical research. Animals harboring this organism may not be acceptable for research involving the liver and gastrointestinal tract. Diagnosis and eventual elimination of this organism from research animals should be a priority.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10475-01 VR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Superovulation and Ova Harvest in Miniature Swine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Victoria A. Hampshire, V.M.D.	Chief, CUUB	CUUB, LASB, VRP, NCRR
John D. Bacher, D.V.M.	Chief, SRU	SRPS, SSB, VRP, NCRR
Axel V. Wolff, D.V.M.	Chief, LASP	LASP, VRP, NCRR
Melissa Yarko-Williams	Biolab. Techn.	CUUB, LASB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Animal Services-Bethesda

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study began as an investigation into the feasibility of using miniature/microswine as animal models for human gene therapy. A need for the development of an animal model for sickle-cell anemia existed within NIH. The standard animal model has been the mouse, although the gene for sickle-cell disease was incorporated but not clinically expressed. Investigators have been able to incorporate this gene into the swine genome in large domestic swine at the United States Department of Agriculture. The use of a miniature swine model would be more advantageous in a metropolitan research institution. The miniature swine breeds (all inbred to date) have had historically a poor reproductive performance. This investigation was undertaken to characterize the ovulatory patterns in synchronized, superovulated microswine of a new outbred variety, the Panepinto micro-pig.

Investigators obtained 15 outbred gilts of the Panepinto outbred strain. The initial results indicate comparable ovulatory yields to domestic stock using standard superovulatory regimes. Synchronization was uniform across the experimental population and achieved reliable consistent estrous patterns. Mean follicle counts in the 15 superovulated gilts was 33 compared to 16 for control animals. The ova harvested from superovulated gilts averaged 27 (81% of follicles).

OBJECTIVES:

To evaluate the feasibility of using micro-sized swine which have historically poor reproductive characteristics as transgenic animal models.

METHODOLOGY:

Fifteen micro-sized outbred gilts of the Panepinto strain were superovulated using conventional synchronization with Altronegest (7ml) for 21 days in the feed. Each gilt was given 1200 IU PMSG SQ 24 hours following the last dose of Altronegest. Forty-nine hours following PMSG treatment the gilts were administered 500 mg HCG IM and checked for standing estrous at 12 hour intervals following HCG administration. All sows were bred 1-3 times in standing heat at 24-48 hours following HCG injection. Sterile surgical harvest via midline laparotomy was performed between 69-71 hours following HCG injection. Ova were harvested via cannulation of the infundibular end of the uterus and flushing retrograde through the fimbrial opening of the oviducts. Ova were counted and determined fertilized following harvest.

MAJOR FINDINGS:

The Panepinto micro-swine demonstrate high reproductive efficiency (nine piglet averages) in the natural setting. In the research laboratory under standard superovulatory regimes, these micro-size swine demonstrate high ovulatory yields which make them suitable models for transgenic work.

SIGNIFICANCE:

The results of this study indicate that a miniature swine model for studies of human disease via transgenic technology is possible and should be considered when there are limiting factors in disease expression among standard rodent models of these diseases.

Animal models of human disease are most valuable in assessing various treatment modalities; especially when critical care procedures are necessitated by the induction of the disease. Chronic venous access and human-like organ systems in the swine model make them particularly attractive animal models for clinical research programs at NIH.

PROPOSED COURSE:

Continuation

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